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The dosage dependent effect exerted by the NM23-H1/H2 homologue NDK-1 on distal tip cell migration in *C. elegans*

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Abstract

Abnormal regulation of cell migration and altered rearrangement of the cytoskeleton are fundamental properties of metastatic cells. The first identified metastasis suppressor NM23-H1, which displays NDPK (nucleoside-diphosphate kinase) activity is involved in these processes. NM23-H1 inhibits the migratory and invasive potential of some cancer cells. Correspondingly, numerous invasive cancer cell lines (e. g. breast, colon, oral, hepatocellular carcinoma and melanoma) display low endogenous NM23 levels. In this review we summarize mechanisms, which are linked to the anti-metastatic activity of NM23.

In human cancer cell lines NM23-H1 was shown to regulate cytoskeleton dynamics through inactivation of Rho/Rac-type GTPases. The *Drosophila melanogaster* NM23 homologue AWD controls tracheal and border cell migration. The molecular function of AWD is well characterized in both processes as a GTP supplier of Shi/Dynamin whereby AWD regulates the level of chemotactic receptors on the surface of migrating cells through receptor internalization, by its endocytic function.

Our group studied the role of the sole group I NDPK, NDK-1 in distal tip cell (DTC) migration in *Caenorhabditis elegans*. In the absence of NDK-1 the migration of DTCs is incomplete. A half dosage of NDPK as present in *ndk-1* (+/-) heterozygotes results in extra turns and overshoots of migrating gonad arms. Conversely an elevated NDPK level also leads to incomplete gonadal migration due to a premature stop of DTCs in the third phase of migration, where NDK-1 acts. We propose that NDK-1 exerts a dosage dependent effect on the migration of DTCs.

Our data derived from DTC migration in *C. elegans* is consistent with data on AWD's function in *Drosophila*. The combined data suggest that NDPK enzymes control the availability of surface receptors to regulate cell-sensing cues during cell migration. The dosage of NDPKs may be a coupling factor in cell migration through modulating the efficiency of receptor recycling.

Introduction

Metastasis suppressors inhibit different steps of metastasis formation without globally influencing primary tumor growth. Current knowledge suggests that the human genome contains around 30 genes encoding proteins displaying metastasis suppressor activity (1). Metastasis inhibitors are either not, or poorly expressed in metastatic colonies. NM23-M1 (non-metastatic clone 23, mouse isoform 1) was identified as the first metastasis suppressor about 30 years ago by Patricia Steeg by comparing expression patterns of invasive and non-invasive mouse melanoma cell lines (2). Although expression of NM23-H1, the human counterpart of NM23-M1 was found to be elevated in primary tumors, downregulation of NM23-H1 expression was observed in multiple examples of metastatic tumors, such as breast, hepatocellular, colon cancer and melanoma (3-5). However, it is important to note that in several cancer types (for example cervical, ovarian, prostate tumors or hematologic malignancies) positive association was reported between NM23-H1 expression and tumor progression (6-9).

NM23 (or also called *NME*, which stands for non-metastatic) gene family members encode nucleoside diphosphate kinases (NDPKs), which were described more than 50 years ago (10) as housekeeping enzymes meriting a few lines of text in most biochemistry journals as 'just' catalyzing the conversion of nucleoside diphosphates to nucleoside triphosphates. Yet, the human genome consists of 10 *NM23* paralogs, which are grouped based on their sequence similarity and NDPK activity. Group I NDPKs (NM23-H1-H4 isoforms) all possess nucleoside diphosphate kinase activity and show high sequence homology to one another, whereas group II homologs (NM23-H5-H9 and retinitis pigmentosa 2 (RP2)) are more divergent in sequence and do not display the above enzymatic activity except for the H6 isoform (11). NDPKs function as homo- and/or heterohexamers, assembled from H1 and/or H2 monomers (reviewed in (12)). However, in some instances (see Muimo review in this series), mixed hexamers do not appear to form when associated with other proteins in the membrane such as the cystic fibrosis protein, CFTR.

NM23 homologues are linked to numerous biological processes such as cell proliferation, differentiation (13,14), cell migration (15), signal transduction, transcriptional regulation (16,17), apoptosis (18) and many aspects of development (reviewed in (19)).

Besides nucleoside diphosphate kinase activity some other molecular activities have been attributed to NDPKs including histidine-dependent protein kinase (histidine phosphotransferase) (20,21), nuclease activity (22,23) and lipid bilayer binding (24-28).

NM23-H1 is known to inhibit the migratory and invasive potential of cancer cells. Although the exact molecular mechanisms underlying these processes remain elusive, several mechanisms are consistent with the anti-metastatic activity of NM23. In this work we briefly summarize the mechanisms whereby NM23 or its homologues influence the migratory potential of cells. First we focus on cell lines, then move to the function of the *Drosophila melanogaster* NM23 homologue AWD in tracheal and border cell migration. Finally, our data derived from DTC (distal tip cell) migration in the model organism *C. elegans* are compared, first of all with AWD's function in *Drosophila* and then with human data.

NM23-H1 efficiently inhibits the migratory and invasive potential of tumor cells

Among many functions ascribed to NM23-H1, its ability to suppress motility and invasiveness of tumor cells is a well-accepted characteristics (29). The anti-metastatic effect of NM23-H1 (and its mouse homologue NM23-M1) was demonstrated using mouse models and different cancer cell lines. Boissan and colleagues showed that crossing of *nm23-M1* knockout mice with a mouse strain in which hepatocellular carcinoma had been induced, resulted in double transgenic mice with a higher incidence of lung metastases (30). The metastasis-suppressive function of NM23 was also confirmed by overexpressing NM23-M1 in highly metastatic K-1735 melanoma cells, where endogenous NM23 expression was low: ectopic expression of NM23 therein resulted in reduction in their metastatic potential (31). Similar results were obtained when invasive breast, colon, oral, and hepatocellular carcinoma and different melanoma cell lines displaying low endogenous NM23 levels were transfected with transgenes encoding the H1 isoform (32-37).

Conversely, silencing of NM23 in non-invasive hepatocellular carcinoma and colon cancer cell lines possessing substantial or high endogenous NM23-H1 levels, led to a metastatic, invasive phenotype by altering cell-cell contacts, migratory potential and major signaling pathways linked to tumor progression. As a consequence of NM23 silencing Boissan and colleagues observed upregulation of MT1-MMP (membrane associated matrix

metalloproteinase), increased Rac1 signaling and activation of MAPK (mitogen activated protein kinase)/SAPK (stress-activated protein kinases) and Akt pathways (38). Although the exact molecular mechanism whereby NM23 regulates cell migration still remains unclear, the above data suggest that NM23-H1 inhibits the activity of Rac1, a pleiotropic regulator of cell motility. Indeed, NM23-H1 was shown to negatively regulate a Rac1-specific nucleotide exchange factor, Tiam1, thereby inhibiting Rac1 activation (39). NM23-H1 was also connected to Rac-Rho activation by reducing transcription of the EDG2 gene, encoding a lysophosphatidic acid receptor (40,41). Db1-1, a specific exchange factor of another Rho-type GTPase, Cdc42, was also identified as a binding partner of NM23-H1. Binding of NM23-H1 to Db1-1 resulted in inactivation of Cdc42 (42).

The role of the *Drosophila* NDPK homolog AWD in the regulation of tracheal morphogenesis and border cell migration

The function of NDPKs in cell migration was best characterized *in vivo* in *Drosophila melanogaster*. The NDPK homolog AWD (abnormal wing disc) shows 78% sequence identity to NM23-H1 and H2 isoforms (43). AWD regulates negatively the migration of tracheal and border cells, by influencing the endocytosis of certain chemotactic receptors driving the above mentioned processes (44,45).

Tracheal development in *Drosophila* is used to model tubular morphogenesis (also termed branching morphogenesis (reviewed in (46,47), see also Muimo in this series of papers). Tracheogenesis starts during early stages of embryogenesis when tracheal placodes are formed through the invagination of specialized ectodermal cells. From these placodes develop the tracheal branches, which then elongate and finally fuse to shape the tracheal network. Branching morphogenesis is interpreted as a series of cell migration events as cell division is completed after placode formation. FGF (fibroblast growth factor)/FGFR (fibroblast growth factor receptor) signaling plays a crucial role in tracheal migration. The ligand, branchless/FGF, which is released by the tissues ahead of the advancing tube, is recognized by breathless/ FGFR expressed on the surface of migrating tracheal cells. AWD influences FGFR levels on these advancing tracheal cell surfaces through recycling of the FGF receptor. Endocytosis of FGFR is regulated by Shibire/Dynamin, an atypical large GTPase, whose GTP supply is mediated by AWD (44). Mechanistically, it was recently shown by Boissan and colleagues that NDPKs fuel Dynamin type GTPases locally by GTP to allow them to work at high thermodynamic efficiency (48).

Interestingly, homozygous (*awd-/awd-*) loss of function mutants and *awd+/awd-* heterozygotes show different phenotypes such that in homozygous individuals, a complete disruption of tracheal tubules with dispersed tracheal cells was noted, whereas in heterozygotes ectopic branch migration was observed (49). We will return to this theme in relation to cystic fibrosis later in the review.

Border cell migration during *Drosophila* oogenesis is an important model of vectorial epithelial cell migration (50). The *Drosophila* egg chamber contains the germ cell complex (oocyte and the nurse cells) surrounded by follicular (epithelial) cells. During oogenesis AWD is expressed in the follicular cells. Border cells - which are a special group of 6-10 epithelial follicular cells – secede from the epithelium and migrate towards the anterior pole of the oocyte, and as a result they form the micropyle (50). AWD is expressed in the follicular epithelial cells, but its expression is downregulated in the border cells, which allows their migration (45). In contrast, overexpressing AWD in the border cells blocks their motility (45). Among other pathways PDGF (platelet derived growth factor receptor) and JAK/STAT signaling are known to drive border cell migration (51-53). AWD regulates the migration of border cells through affecting receptor levels on the cell surface via internalization of the VEGFR (vascular endothelial growth factor receptor)/PDGFR homolog Pvr and the JAK/STAT receptor homolog Domeless in cooperation with Shi/Dynamin (45,51,52). The mechanism whereby AWD regulates Pvr and Domeless receptor levels on border cell surfaces is similar to that in tracheogenesis (see above). Thus we have an emerging paradigm that a key regulator of nucleotide balance is also regulating receptor residence time.

The idea of NDPK's dosage dependence comes from *Drosophila*: AWD functions in the follicular epithelial cells of the egg chamber in a dosage-dependent manner

The Shearn laboratory showed first that AWD is expressed and functions in the follicular cells of the egg chamber during oogenesis (54). Next, the Hsu laboratory demonstrated that AWD is required to maintain the epithelial integrity of follicular cells by regulating the turnover of adherens junction components (55). AWD exerts this effect through its endocytic controlling function towards multiple adherens junction components such as E-cadherin, beta-catenin and alpha-spectrin.

In addition, Hsu and colleagues examined how lack and overexpression of AWD affect the morphology and structure of follicular epithelial cells. The absence of AWD in these cells

resulted in an abnormal epithelial structure: because of spreading of adherens junction components, the cells accumulated and piled up. On the other hand, as a consequence of excess AWD follicular cells lose adherens junction components from their surface, adopted a spindle-like morphology and underwent a morphological change reminiscent of EMT (56). These data first showed that both lack or have an excess AWD activity disturb epithelial integrity and suggested that an optimal dosage of AWD is needed to balance demand and supply of adherens junction components in follicular cells (49).

The dosage dependent effect of NDK-1 exerted on distal tip cell (DTC) migration in *C. elegans*

Consequently, we investigated the potential dosage dependent effect of NDK-1, the nematode group I homologue NDPK (57) exerted on cell migration, especially on the migration of distal tip cells (DTCs) in *Caenorhabditis elegans*.

NDK-1, the sole *C. elegans* group I NDPK, shows 65 % identity and 85% overall similarity to NM23-H1 and H2 isoforms (57). As group I NDPKs are well known negative regulators of cell migration, first we intended to examine how the conserved worm protein, NDK-1 functions in a human environment, using highly invasive MDA-MB231T breast carcinoma cells as a model system, where endogenous NM23 level is low (40). Therefore MDA-MB231T cells were transfected with worm NDK-1, NM23-H1 and H2, respectively, and the motility of the transfected lines was examined (58). We found that the migratory potential of MDA-MB231T cells was significantly suppressed, when NDK-1 or its human counterparts were overexpressed. These data show that NDK-1 acts in a conserved manner in cell migration and that *C. elegans* serves as a tractable genetic model to study the biological functions of NDPKs.

Caenorhabditis elegans serves as a useful and simple model system to investigate the process of cell migration. Distal tip cells (DTCs) are responsible for gonadal morphogenesis (reviewed in (59): these cells of somatic gonadal origin generate the shape of the gonad. DTCs are born in L1 larval stage and are located at the distal edges of the gonad primordium (Figure 1A). In L2 stage one of the two DTCs moves anteriorly, the other posteriorly along the ventral body wall muscles, resulting in an elongated gonad. In L3 larval stage each DTC turns 90 degrees and moves to the dorsal muscles. After a second turn DTCs migrate dorsally to the midbody region in L4 larvae and finish their migration in adulthood dorsal to the vulva.

In adult hermaphrodites this migratory path results in two symmetric U-shaped gonad arms (59,60), which can be easily followed during development by DIC microscopy due to transparency of the worm (Figure 1B).

Hence, we examined the role of NDK-1 in gonadal migration in the worm. First we characterized homozygous *ndk-1(-/-)* loss-of-function mutants and found that these nematodes display an abnormal gonad shape. In addition, worms transgenic for NDK-1::GFP showed expression in the distal tip cells. The above data indicated that NDK-1/NDPK plays a role in DTC migration. Detailed analysis of homozygous *ndk-1(-)* mutants revealed that in the absence of NDK-1 the migration of DTCs is incomplete, results in J-shaped gonad arms in the majority of mutants due to a premature stop of DTCs in the third (dorsal) phase of migration (58); Figure 1D).

This finding was interesting but perhaps surprising, because by knocking out a group I NDPK homologue we would expect enhanced/ectopic migration of DTCs instead of incompletely developed gonad arms, based on the well known inhibitory effect exerted on cell migration by this gene family. However, overexpression of NDK-1 in highly invasive breast carcinoma cells caused inhibition of the migratory potential similar to NM23-H1 and H2. One possible explanation would be that NDK-1 affects cell migration in opposing ways in different cellular environments (e.g. it promotes migration of DTCs in the worm but inhibits cell migration in human cell lines) similar to *Dyctiostelium*, where NDPK was shown to influence growth in an opposing manner depending on a spatial cellular context of axenic growth in broth versus spreading growth on a surface (61).

To better understand NDK-1's function in the worm, we further investigated how different levels of NDK-1 expression affect the migration of DTCs: in order to complete our dataset we analyzed DTC phenotypes in *ndk-1(+/-)* heterozygotes and NDK-1 overexpressing transgenic worms.

In outwardly normal wild-type *ndk-1(+/-)* heterozygotes, a half dosage of NDK-1 protein is expressed. In the majority of heterozygous worms the reduced level of NDK-1 did not interfere with gonad morphogenesis, as in 76% of the cases wild-type gonad arms were observed (n=66). However, ectopically migrating DTCs were found in 24% of the observed gonad arms (n=66) indicating that in these worms one single functional copy of the *ndk-1* gene does not produce sufficient NDK-1 protein to generate wild type U-shaped gonad arms (haploinsufficiency with 24% penetrance). Ectopically migrating gonad arms manifested either in an overshoot (5%) or an extra turn (19%) phenotype (Figure 1C). Overshoot occurs

when the gonad elongates past the vulva, extra turn means that the migratory path of DTC includes more than one turn.

In order to examine the effect of excess NDK-1 exerted on DTC migration, a transgenic strain overexpressing NDK-1 was generated by ballistic transformation. The transgene used for transformation contained a $p_{\text{hsp-16.2}}$ NDK-1::mCherry cassette, where NDK-1::mCherry expression is driven by a heat shock promoter. Unfortunately we could not generate an integrated transgenic line, but in our strongest non-integrated line 90% of the animals carried the transgene as extrachromosomal arrays, thus these animals showed NDK-1::mCherry expression in response to heat shock. After heat shock treatment, 14% of mCherry-positive worms (n=42) showed incompletely elongated, J-shaped gonad arms (Figure 1E). The low penetrance of the observed phenotype is likely due to mosaicism of the transgene.

Conclusions

NM23-H1 is known to inhibit the migratory and invasive potential of cancer cells.

Numerous invasive cancer cell lines (e. g. breast, colon, oral, hepatocellular carcinoma and melanoma) display low endogenous NM23 levels. In independent experiments using different lines the migratory potential of these cells became significantly lower when transfected with transgenic, exogenous NM23 (32-37,62). In the opposite scenario, silencing of NM23 in previously non-invasive colon cancer and hepatocellular carcinoma cell lines (expressing higher level of NM23-H1) resulted in an invasive phenotype with altered cell-cell contacts, activation of signal transduction pathways related to tumor progression (38), which are hallmarks of EMT. Some data link the function of NM23-H1 to different members of Rho GTPases, which play an essential role in cell migration and invasion by regulating dynamics of the cytoskeleton. For example NM23-H1 was shown to interact specifically with Tiam1 and Dbl-1, cofactors of Rac1 and Cdc42 respectively, thereby inhibiting the activity of these small GTPases (39,42).

Cystic Fibrosis patients have a higher risk of cancer such as colon cancer with some teenagers even developing metastasis. CDC-42 and the CF protein CFTR have also been linked (63), which is interesting in the context of the binding of both NDPK H1 and H2 in the CFTR hub of proteins, that include potassium channels linked to NDPK function and cell membrane turnover during cell migration has also been reported (cited in Muimo, this series of reviews).

Robust evidence was provided by researchers working on the famous model organism *Drosophila* that the NM23 homolog AWD functions together with dynamin in endocytosis in a highly specific manner (64). AWD is an inhibitor of cell migration in tracheal morphogenesis and border cell migration (44,45). In cooperation with Shi/Dynamin, AWD/NDPK suppresses cell motility by downregulating chemotactic receptor levels on the cell surface through receptor internalization.

Our group studied the role of NDK-1, the sole *C. elegans* group I NDPK homologue in the process of distal tip cell (DTC) migration. Homozygous *ndk-1(-/-)* loss of function mutants showed predominantly incompletely elongated J-shaped gonad arms (58).

To better understand the function of NDK-1 in DTC migration, we aimed to fill the missing gaps in our dataset: we observed DTC migration patterns in *ndk-1+/ndk-1-* heterozygotes and generated an *ndk-1* overexpressing strain in order to analyze DTC migration phenotypes caused by excess NDK-1. In *ndk-1+/ndk-1-* heterozygotes, in 24% of the cases ectopically migrating gonad arms (extra turn and overshoot phenotypes) were scored, whereas in nematodes overexpressing NDK-1 again incompletely migrating gonad arms were detected with 14% penetrance (see summarized in Table 1).

The above data suggest that different NDK-1 protein levels lead to diverse DTC migration phenotypes: in the absence of NDK-1 DTC migration is incomplete, wild-type dosage of the *ndk-1* gene leads to normal U-shaped gonad arms, half dosage of *ndk-1* present in heterozygotes results in extra turns and overshoots of migrating gonad arms, and in response to excess NDK-1 incompletely elongated, J-shaped gonad arms are developed (Table 1).

In *Drosophila* during tracheal morphogenesis, homozygous *awd-/awd-* loss of function mutants and *awd+/awd-* heterozygotes show also different phenotypes: in homozygotes a complete disruption of tracheal tubules, whereas in heterozygotes ectopic branch migration was observed (49), Table 1). In our opinion, a parallel can be drawn between DTC migration phenotypes in the nematode and tracheal migration phenotypes in the fruit fly: complete absence of the NDPK homologue results in incomplete migration, but presence of half dosage of NDPK causes ectopic migration in both models (e.g. extra gonadal turns in the worm and ectopic tracheal branches in the fly, see Table 1). We note that in cystic fibrosis, where NDPK H1 and H2 are defective in activity and complex formation with other partners, tracheal formation is defective in some babies(65).

In *Drosophila*, overexpression of AWD was studied in the process of border cell migration, where excess AWD resulted in stalled migration of border cells (Table 1). In the nematodes,

we overexpressed NDK-1 in the DTCs using a transgenic strain for NDK-1, driven by a heat shock promoter and demonstrated insufficiently elongated gonad arms (Table 1). In both cases overexpression of NDPK caused an inhibition of cell migration: 1. in the fly border cells did not reach their final position in the egg chamber (e.g. they did not arrive to the oocyte), 2. in nematodes the migration of DTCs was blocked at the dorsal phase of gonadal migration where NDK-1 functions.

In *Drosophila*, the mechanism whereby AWD influences tracheal and border cell migration is well known: AWD works as a local GTP supplier of Shi/Dynamin, which regulates chemotactic receptor levels on the surface of migrating cells (e.g. FGFR levels in case of tracheal cells and Pvr levels on border cells) through receptor internalization by its endocytic function (44,45).

In *C. elegans*, the mechanism whereby NDK-1 influences DTC migration is not yet known, further investigations are needed to better understand its function in this process.

However, DYN-1, the Dynamin worm homologue was shown to function in DTC migration (66), in addition our group identified a genetic interaction between NDK-1 and DYN-1 (58), suggesting that an NDK-1/DYN-1-mediated endocytic mechanism might regulate chemotactic receptor levels, which play a role in DTC migration (Figure 2).

Integrin receptors are known to act in the dorsal/ third phase of DTC migration similar to NDK-1 (58,67). The loss of function mutant phenotype (J-shaped gonad arms) of *ndk-1* is reminiscent of that of *pat-3*, which encodes a worm beta-integrin (66). Thus, beta-integrin is a good candidate as a receptor present on distal tip cells, whose cell surface level might be modified by NDK-1/DYN-1 activity.

Taken together, our aim was to examine how the absence, the half dosage of NDK-1 and excess NDK-1 influence the migration of distal tip cells (DTCs) in *C. elegans*. We analyzed how different levels of an NDPK affect the migration of the same cell type, a single gonadal leader cell, and found that NDK-1/NDPK has a dosage dependent function in DTC migration (Table 1). The data suggest that some parallels can be drawn between DTC migration phenotypes in the worm and tracheal and border cell migration phenotypes in the fly, emerging in response to different NDPK protein levels (Table 1). In *Drosophila*, receptor internalization by Shi/Dynamin mediated endocytosis is confirmed as a mechanism whereby AWD influences cell migration. In *C. elegans* NDK-1's exact molecular function is not known yet during DTC migration but a DYN-1/NDK-1 mediated endocytosis might play a role in internalization of receptors, such as netrins or integrins that are known to drive the migration of these gonadal leader cells (67) (Figure 2). This issue needs further investigation.

NDPKs function as exclusive GEF-like factors to fuel Dynamin GTPases by GTP to allow them to work with high thermodynamic efficiency in membrane rearrangements (48). In the absence of NDPKs, Dynamin is not fuelled efficiently by GTP and decreased Dynamin activity leads to reduced membrane invagination, which might have a consequence on cell surface receptor levels on migrating cells through receptor recycling. To explain how the dosage of NDPKs might influence cell migration, extensive work in *Drosophila* provides examples. Loss of AWD function was examined in tracheal cells of homozygous and heterozygous *awd* mutants, and an over accumulation of BTL (breathless)/FGFR was detected on the surface of tracheal cells (44). Similarly, knock-down of *awd* in cultured *Drosophila* S2 cells resulted also in an elevated FGFR level on the cell surfaces (44). Different tracheal phenotypes were observed in case of *awd* homozygotes and heterozygotes: in the former case a complete disruption of tracheal tubules, whereas in the latter case ectopic branch migration was noted (44,49). In homozygotes complete loss of AWD resulted in over accumulation of surface receptor and delayed migration, which is the net outcome of random movement of the cells as a consequence of lack of directional cues due to symmetrical signal activation of the receptor. In heterozygotes the former severe phenotype is rarely seen, in the majority of cases the overall tracheal network is developed often with ectopic branches.

Low level of AWD allows border cells to migrate from the epithelium to the anterior pole of the oocyte during normal oogenesis (45). Overexpression and knock-down of *awd* was analyzed by the Hsu lab in the process of border cell migration, where both conditions resulted in stalled migration of border cells (45).

Ectopic expression and lack of AWD led to similar phenotypes (stalled migration), however influencing the level of Pvr, the chemotactic receptor on border cell surfaces in an opposite way: AWD overexpression resulted in reduced Pvr levels, while its knock-down increased the expression of the chemotactic receptor.

Consistently, earlier work have shown that both lack and excess of Pvr signaling impair border cell migration: 1. overexpression of Pvr results in spinning of border cells without moving forward due to suppressed, nondirectional chemotactic signaling response 2. *pvr* loss of- function border cells do not move, as lack of chemotactic receptors leads to downregulated Pvr signaling (51,52).

We also examined the effect of the worm group I NDPK, NDK-1 in a heterologous system: overexpression of NDK-1 in metastatic MDA-MB231T breast carcinoma cells resulted in inhibition of the migratory potential similar to NM23-H1/H2. Thus, the worm NDPK was able to replace its human counterparts in breast carcinoma cells. However the mechanism

whereby NDK-1 exerts its effect remains to be determined, as in human cell lines H1 and H2 isoforms were shown to act in several different mechanisms which result in inhibition of migratory and invasive potential of breast carcinoma cells.

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